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Cluster Analyses of *Pterocarpus indicus* (Narra) Leaf Shape in Cebu, Philippines

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***Abstract*: *Pterocarpus indicus*, commonly known as narra, is a significant tree in the Philippines, environmentally, economically and medically. This paper analyzes the leaf shape of the tree within the province of Cebu, where three sampling sites were identified, namely: northern, central and southern Cebu. 150 leaves per sampling site that contain seven leaflets were the subject of the analyses using the software SHAPE version 1.7. Generated eigenvalues of its first and second principal components were used for cluster analyses. Results showed that except for right leaflet-3, the northern and southern samples were closely clustered than those from the central. Even the average leaf shape is consistent with the leaflet shape. The difference of the shapes can be attributed to the tree's phenotypic plasticity since climatic data (temperature, precipitation, humidity) and elevation differs from the sampling sites, thus making the tree a potential indicator of environmental changes. A more intense shape analyses that considers varying leaflet number, age of tree, spatial distribution and anthropogenic disturbances are highly recommended for future studies.**

***Keywords*: *Pterocarpus indicus*, SHAPE, leaf plasticity, Cebu.**

I. INTRODUCTION

Elliptic Fourier Analyses (EFA) has been used by several biologists in quantifying biological shapes [1]. The mathematical aspect of the technique has been popularized by Kuhn and Giardina in 1982 [2]. In their paper, they described that Elliptic fourier simplify the contours of images with convenience and high accuracy. Since then, EFA slowly gained grounds in its application in biological shapes. Haruta (2011) acknowledge that EFA is a powerful tool that can analyze high morphological variability [3].

In taxonomy, EFA has been utilized to settle disputes in biological classifications. One example is the work of Zhan and Wang (2012) [4]. Here they settled an argument regarding antlion taxonomy, where they concluded that the current antlion classification is consistent with their findings. The technique has also been employed in anthropological studies, where Ferrario et al. [5], studied dental morphologies in comparing the first permanent molars among their subjects.

EFA has also been used to map and identify weed species, where researchers have found out that the analyses can generate data for identification at 93.6% accuracy using leaf shapes, even during germination [6]. In the other hand, the method can also expose some inconsistencies in the molecular data of the species and that of its morphology when analyzed taxonomically [7].

Yoshioka et al. [8] in their studies on petal shapes concluded that the macroenvironment have bigger influence on plant shape than the plant's genotypes. Phenotypic plasticity, or the morphological change in response to the environment, is important in observing changes in the environment [9]. Stationary organisms such as plants are less likely to migrate to find more appropriate environment that suits best to its physiologic needs in the event of climate changes [10]. Sexton et al. [11] reasoned that the capacity of plants to exhibit plasticity allowed them to have wider environmental tolerance which give them a more superior ability to colonize various types of habitats. Even large scale changes in local climate, plants, specifically trees, were traced to possess considerable plasticity [12]. Trees that undergo long-term physiological adjustments with respect to a changing environment, say global warming, require different numbers of generation before the species can fully adapt to its environment. However, this generation requirement differs from one geographic location to another [13].

On the other hand, Richards et al. [14], together with those working on invasion science have described phenotypic plasticity having an important role in successful invasion of plants in a given area. On their review paper, they were able to derived two hypothesis for this attribute. First, native and non-invasive species tend to be less plastic than invasive species. Secondly, populations of invasive species introduced in a given area have been considered to have evolved more plasticity than those that are native

Though plasticity has been observed to various plant species, it also have its limits. The limiting factors can be abiotic or biotic. For example, Valladares et al. [15] found that plant populations with higher plasticity response to light are those that have lower survival rates when light or temperature is limited. The adaptive capacity of plants can serve as a very important predictor on the magnitude of the effects of climate change [16].

Pterocarpus indicus is native to several tropical and subtropical regions, specifically in the north and southwest Pacific regions, and from East and Southeast Asia. It grows up to 35 m and its canopy broadens in open spaces. The tree usually thrives in closed and secondary forests, though in also grows in riverine. It also in elevation up to 1300 m. Its ecological uses include stabilization of soil and is well adapted to several atmospheric disturbances [17].

In the Philippines, *P. indicus* is commonly known as narra. Its common uses includes furniture, ornaments, medicine and food. These trees are regular sights along the highways and other establishments of Cebu City and can also be found in Cebu province’s southern and northern regions.

The tree’s compound leaves usually contain five, seven and nine leaflets. And its leaflet shapes analyses with environmental connections have been poorly studied. Hence this paper would quantitatively analyze leaflet morphology from *P. indicus* in central, south and north Cebu using its shapes.

II. MATERIALS AND METHODS

A. Sampling Sites:

To represent the entire province of Cebu, three major areas were identified: Municipalities of Carmen and Catmon and Danao City (north), Cebu City (central), and Municipalities of San Fernando and Argao (south). Table 1 summarizes the coordinates of the sampling sites and its respective elevation.

TABLE 1. Coordinates of the sampling areas with its specific sampling sites

Sampling Site	Sampling Area	Coordinates
North	1	10˚37’54.9’’ N 124˚1’40’’ E
	2	10˚36’9’’ N 124˚1’21’’ E
	3	10˚36’11’’ N 124˚1’29’’ E
Central	1	10˚18’6’’ N 123˚53’50’’ E
	2	10˚18’2’’ N 123˚53’52’’ E
	3	10˚17’55’’ N 123˚53’50’’ E
South	1	9˚53’10’’ N 123˚35’54’’ E
	2	10˚9’38’’ N 123˚42’19’’ E
	3	10˚9’38’’ N 123˚42’19’’ E

B. Leaf Sampling:

Mature and healthy, seven-leaflet *P. indicus* leaves were the subject of the study. In every area, 150 leaves were collected (15 per tree). Leaflets were then immediately cut and segregated based on their placement that was hypothetically assigned: (Fig. 1). Right after leaflet segregation, leaflets of the same placement were positioned at the same orientation and with the upper epidermis facing downwards.

All sampling was conducted on the third week of April 2017.

C. Image Enhancements:

Scanned images were converted into bitmap format to meet the compatibility requirements of the software used in the analyses.

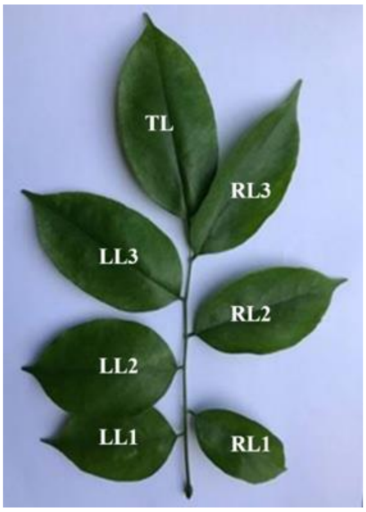

Fig. 1. Hypothetical labeling of leaflet placement. (LL-left leaflet; RL-right leaflet; TL-terminal leaflet)

D. Elliptic Fourier Analyses:

In analyzing the images, the software SHAPE version 1.7 [18], a program package that evaluates biological shapes based on elliptic fourier descriptors (EFD), was used. The first two eigenvalues of its principal components were subjected for cluster analyses using Paleontological Statistics (PAST) software version 2.17 [19].

III. RESULTS AND DISCUSSION

Generating the average leaf shape between the three sampling sites, as shown in Fig. 2, shows that north and south Cebu tends to cluster more at the center. On the other hand central Cebu tend to be more scattered. This means that leaflet shapes in the northern and southern part of Cebu are much similar, while that of central Cebu are varied (consider Figure 4). This is then consistent with the individual leaflet analyses.

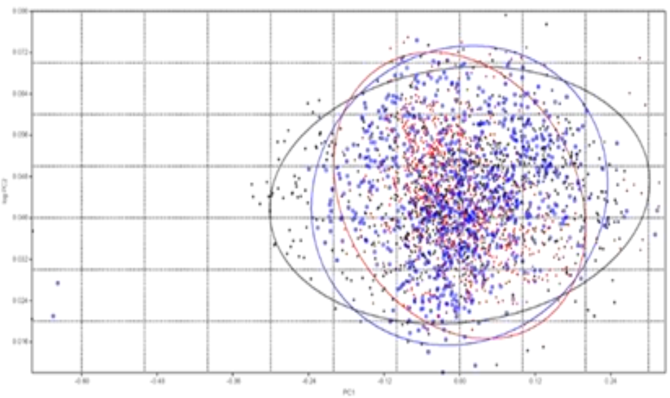

Fig. 2. Cluster analysis of the overall leaf shape between the three sampling sites. (Black/dots-Cebu City; Blue/square-Cebu south; Red/cross-Cebu north)

Cluster analysis, based on the generated eigenvalues, of the different leaflets of 5-leaflet *P. indicus* in the province of Cebu showed that except from right leaflet-3 (Fig. 3F), samples coming from the northern and southern part of the province are clustered closer compared to those from Cebu City (Fig. 2).

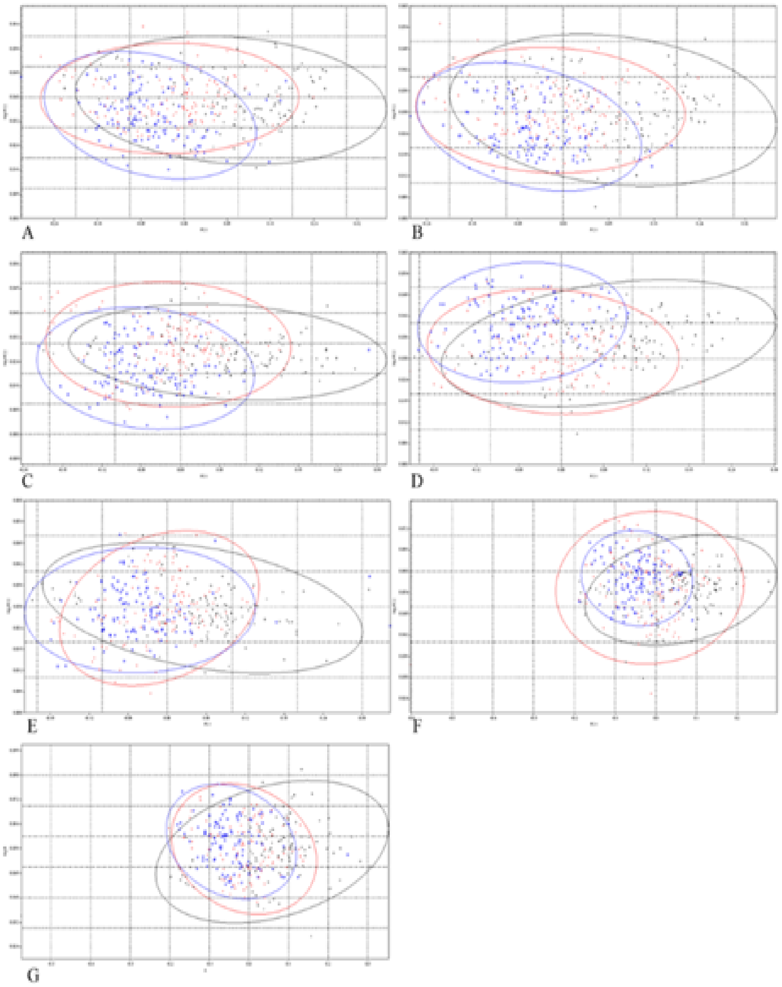

Fig. 3. Cluster analysis of the different leaflets of *P. indicus* within Cebu province. (A-left leaflet 1; B- right leaflet 2; C-left leaflet 2; D- right leaflet 2; E-left leaflet 3; F-right leaflet 3; G-terminal leaflet.)(Cluster ellipse: Black/dots-Cebu City; Blue/square-Cebu south; Red/cross-Cebu north).

The close clustering between the northern and southern regions can be attributed to the plant's phenotypic plasticity. Plasticity is more common in immobile organisms such as plants. Plants' leaves are most likely to adjust to environmental factors that triggered plasticity. In their review paper, Nicotra et al. [20] stipulated that plasticity is much needed as a response to environmental changes. They also state that understanding how plants respond to these changes is important especially to crops plants and native species.

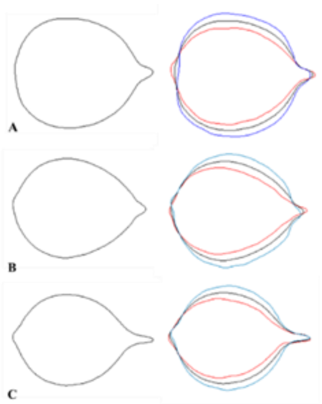

Fig. 4. Average leaf shapes of the sampling sites with its -2StD (red) and +2StD (blue). (A-Central Cebu; B-North Cebu; C-South Cebu)

TABLE 2. Elevation of the sampling sites and its averages

Sampling Site	Sampling Area	Elevation (m)	Average (m)
North	1	3.0	10.37
	2	14.8	
	3	13.3	
Central	1	13.3	11.57
	2	14.0	
	3	7.4	
South	1	1.0	695.67
	2	1043.0	
	3	1043.0	

This phenotypic plasticity can be attributed to several factors. Elevation levels where these samples were taken vary from one site to the other. In Table 2, northern and central Cebu have average elevations of 10.37 m and 11.57 m respectively. On the other hand, south Cebu has an average of 695.67 m. Average climatic conditions three months prior to the sample days showed that temperature does not vary in central and northern Cebu. However, south Cebu is relatively having lower temperature. As the first quarter ends, precipitation decreases on central and southern Cebu, and not on the northern part. By average, northern and southern Cebu tends to have higher precipitation rate than central Cebu. Lastly for humidity, north and south Cebu have slightly higher humidity percentage especially in the first two months of the year (78%) compared to central Cebu (77%). Humidity is similar to the three sites in March (74%) (Table 3).

TABLE 3. Average climatic data on the sampling sites showing temperature, precipitation and humidity

Climatic Factors	Months	Central	North	South
Temperature (°)	January	30	30	26
	February	30	30	27
	March	32	32	27
Precipitation (mm)	January	79	79	129
	February	65	83	93
	March	6	6	91
Humidity (%)	January	77	78	78
	February	77	78	78
	March	74	74	74

Central Cebu's high variability could also be attributed to its precipitation rate. As shown in Table 3, precipitation in this area is lower compared to other sampling sites. *P. indicus* in this area were intentionally planted as they were regular view within the city streets. Since central Cebu have lower humidity and that humidity and precipitation are positively correlated, hence precipitation is expected to be lower. Unlike central Cebu, the northern and southern sampling sites' most P. indicus are growing within secondary forests. Different phenotypic plasticity responses were observed in various plants exposed to different amount of precipitation [21], [22], [23], [24], [25].

Fraser et al. [26] hypothesized that in *Pseudoroegneria spicata* phenotypic plasticity is an adaptive mechanism that affects several aspects such as density in stomata, biomass and leaf area. This is in response to changing water levels and the increase in temperature. In another plasticity study in relation to environmental factors, Vitasse et al. [27] discussed that plants in the lower altitude tends to have increased rate of senescence especially when associated with elevated temperature. In another altitude-related plasticity study concerning a Hawaiian tree species *Metrosideros polymorpha*, Cordell et al. [28] identified that there is a decreased in morphological features including size of leaves, internode length and petiole length as elevation increased. However, when planted in a common garden, they noted that these features where retained. They therefore suggest that there could be a genetic basis that can be responsible for this difference in the tree's response in relation to altitude, allowing the species to have wider ecological distribution.

Lastly, another possible factor that accounts to a closer cluster in the northern and southern part of Cebu is humidity. Plants growing in less humid and drier conditions tend to have increased xeromorphosim, or the plant's structural adaptation, allowing changes in features in the leaves' parenchyma, schlerenchyma, gelatinous fibers, and tannin abundance [29]. Resin availability in leaves are also more pronounced in a humid environment than in a drier regions [30].

IV. CONCLUSION

The variability of leaf shape of *P. indicus* found in the province of Cebu is caused by the interplay of various environmental factor such as elevation and climatic conditions. This just shows that *P. indicus'*s leaves and its plasticity capacities are possible indicators of environmental changes within the province and its quantitative description is significant to further understand the physiological behavior of the Philippine tree ambassador. Though phenotypic plasticity is more favored in this paper, its genetic counterpart is not ruled out since it has a significant role of species' population survival especially in a fast changing environmental features [31].

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